Figure 1

Allosteric Effector	Structure or Name
PCHA-DPG	Penta-cyclohexylammonium 2,3-diphosphoglyceric acid
5Na-DPG	Penta-sodium 2,3-diphosphoglyceric acid
IHP	Inositol hexaphosphate
CHA	Cyclohexylammonium
CHA-IHP	Cyclohexylamine added to IHP to give a solution with a pH = 7.1-7.4

Figure 2

Effector	P_{50} CONTROL WB mmHg	P_{50} EFF: WB mmHg	CONC. EFF mM	CONC EFF:WB mM	OSMOL. EFF mOsM	pH EFF.	pH EFF:WB or EFF:*fHb*	Volume Ratio EFF:WB
HBS+					310		7.22	
***f*Hb in HBS+**							7.22	
PCHA-DPG								
WB	37	55.5	30	22	205		7.42	1:0.375
WB	25	36	30	22	221	7.8	7.33	1:0.375
WB	37	37	30	22	313		7.12	1:0.375
WB	37	37	30	12	317		7.02	1:1.5
WB in Bis-Tris	37	38.2	30	22	341		7.1	1:0.375
WB	37	36	30	12	310		6.8	1:1.5
5Na-DPG								
WB	37	38.2	30	22	163		7.4	1:0.375
WB	37	39.5	30	22	321		7.1	1:0.375
IHP								
WB	37	38.2	30	22	185		7.3	1:0.375
*f*Hb	16	40.5					7.19	0.25 µM EFF
CHA								
WB	26.8	28.5	30	22	220		6.23	1:0.375
WB	26.8	26.8	30	22	245		6.75	1:0.375
CHA-IHP								
WB	26.8	42	30	22	220		6.36	1:0.375
WB	24.7	58.2	25	14.3	171		6.93	1:0.35
*f*Hb	16	53					7.17	0.25 µM EFF

*f*Hb = free hemoglobin; WB = whole blood; EFF = effector.

Figure 3

Sample	Observed O_2 P_{50} (torr)
Human whole blood	9.3 (pH 7.47)
Washed Goldfish blood cells	20.0 (pH 7.52)
Human *f*Hb	4.7 (pH 7.1)
Goldfish *f*Hb	8.5 (pH 7.1)
Goldfish *f*Hb + 0.25 µmol IHP	15.0 (pH 7.1)
Goldfish *f*Hb + 0.5 µmol PCHA-DPG	10.3 (pH 7.1)
Goldfish *f*Hb + 0.5 µmol ATP	21.0 (pH 7.08)
Goldfish *f*Hb + 0.5 µmol GTP	23.0 (pH 7.11)

The data presented in this figure was acquired at 25 C in Bis-Tris buffer at pH 7.2-7.4.

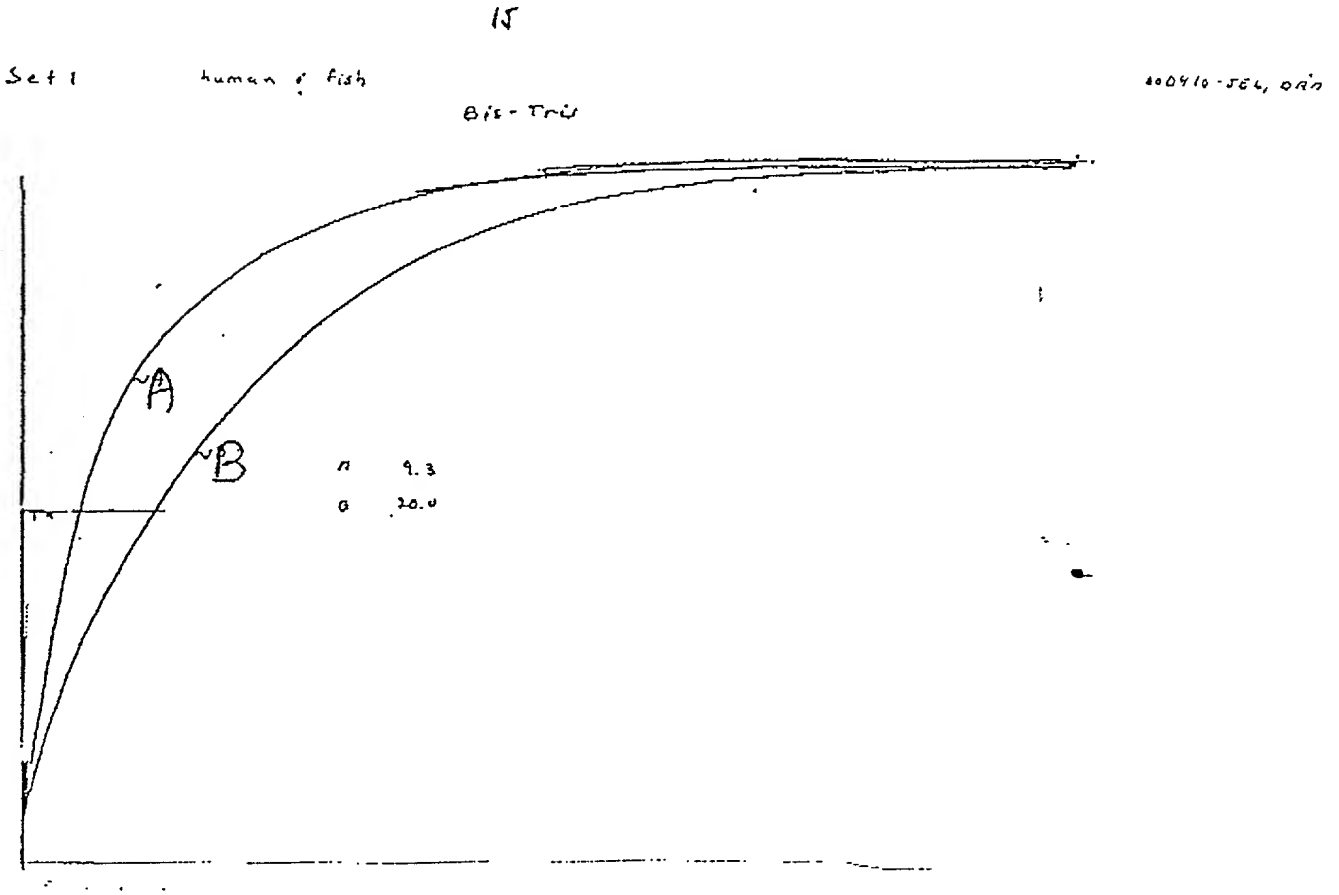
Figure 4

A:	Human whole blood	pH 7.47	$P_{50} = 9.3$
B:	washed goldFish blood	pH 7.52	$P_{50} = 20.0$

All experiments were in Bis-Tris buffer and 25 °C.

Collection methods for blood from up to 20 goldfish requires higher amounts of anticoagulant than from a single human source. From published protocols, a special washing at 4 °C and additional steps to remove nucleic acids from lysed red cells are required.

Obviously the O_2 dissociation for Fish hemoglobin entrapped within a red cell is optimal at a lower temperature than Human .



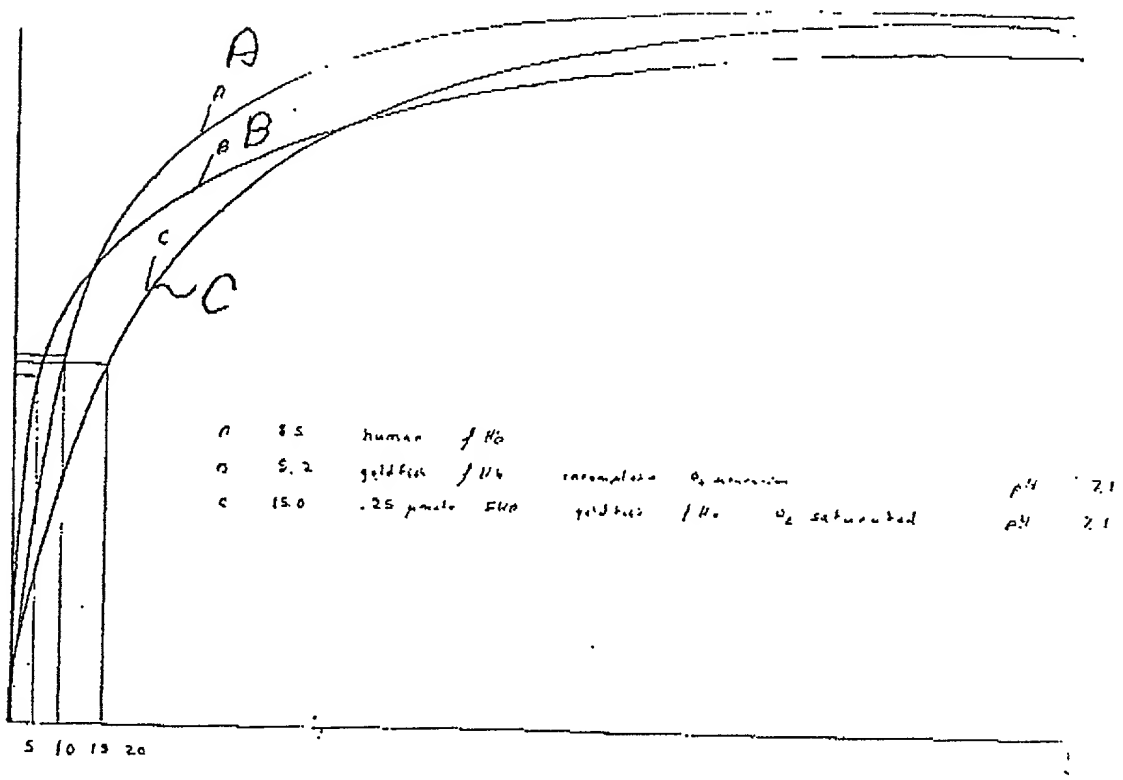


Figure 5

The experiment an figure 4 were recorded at a pH optimal for humans. The pH for fish studies is generally lower, pH 7.1 versus 7.4. Previously we have reproduced data showing the strong pH dependence for human *free* hemoglobin at different pH. These experiments (figs. 10 and 11 are at pH 7.1. Because of variations with isolating fish red cells and the temperature coefficient on pH illustrate these effects. Previously I had acquired some familiarity with HEPES Buffered and adjusting for its temperature coefficient, they did not translate to Bis-Tris buffers.

A:	human *free* hemoglobin	pH 7.1	$P_{50} = 4.7$
B:	goldfish *free* hemoglobin	pH 7.1	$P_{50} = 8.5$
C:	sample B +0.25 µmol IHP	pH 7.1	$P_{50} = 15.0$

All experiments were in Bis-Tris buffer and 25 °C.

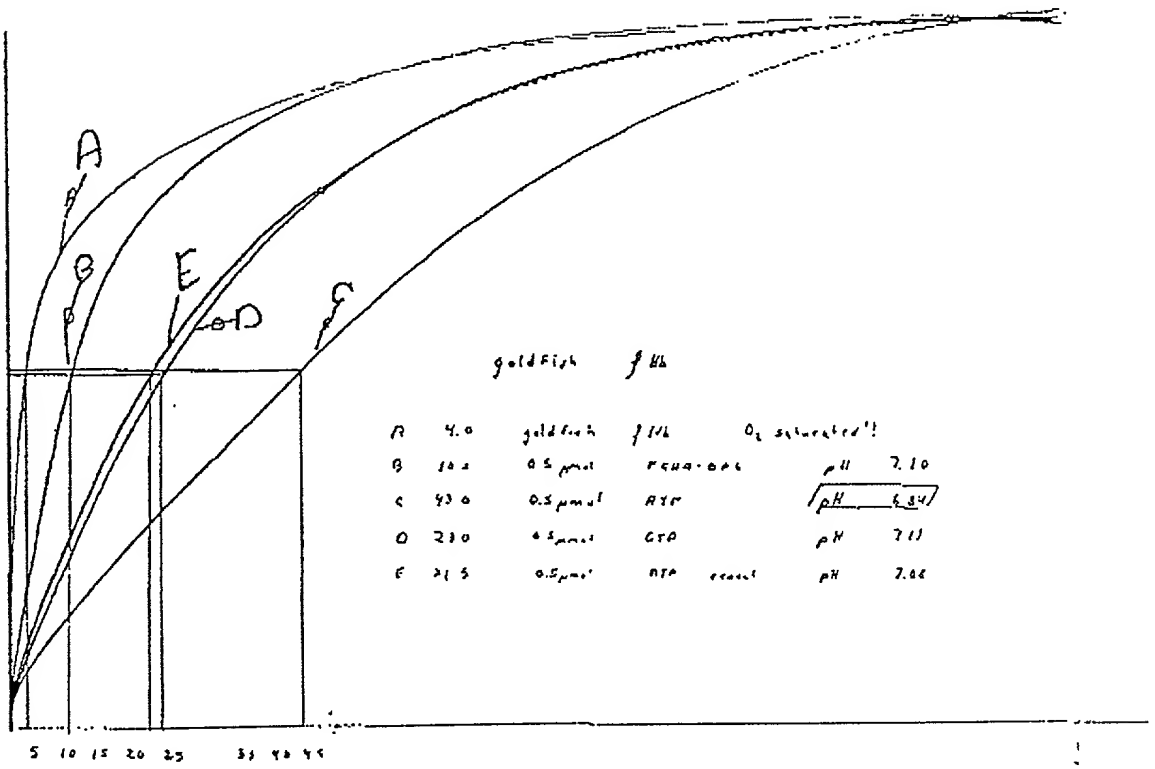


Figure 6

A:	goldfish *free* hemoglobin	pH unk*	$P_{50} = 4.0$
B:	sample A +0.5 µmol PCHA•DPG	pH 7.1	$P_{50} = 10.3$
C:	goldfish *free* hemoglobin + 0.5µmol ATP	pH 6.84**	$P_{50} = 43.0$
D:	goldfish *free* hemoglobin + 0.5µmol GTP	pH 7.11	$P_{50} = 23.0$
**E:	goldfish *free* hemoglobin + 0.5µmol ATP	pH 7.08	$P_{50} = 21.0$

All experiments were in Bis-Tris buffer and 25 °C.

* pH measured after the addition of PCHA•IHP in ccuveve A. All other samples began with a new aliquot of *free* fish hemoglobin prior to the addition of the allosteric EFFector.

** After adding the ATP the pH of the sample (Run C) was too low. The pH of the ATP was adjusted to yield an appropreiate PH after addition to the sample, Run E

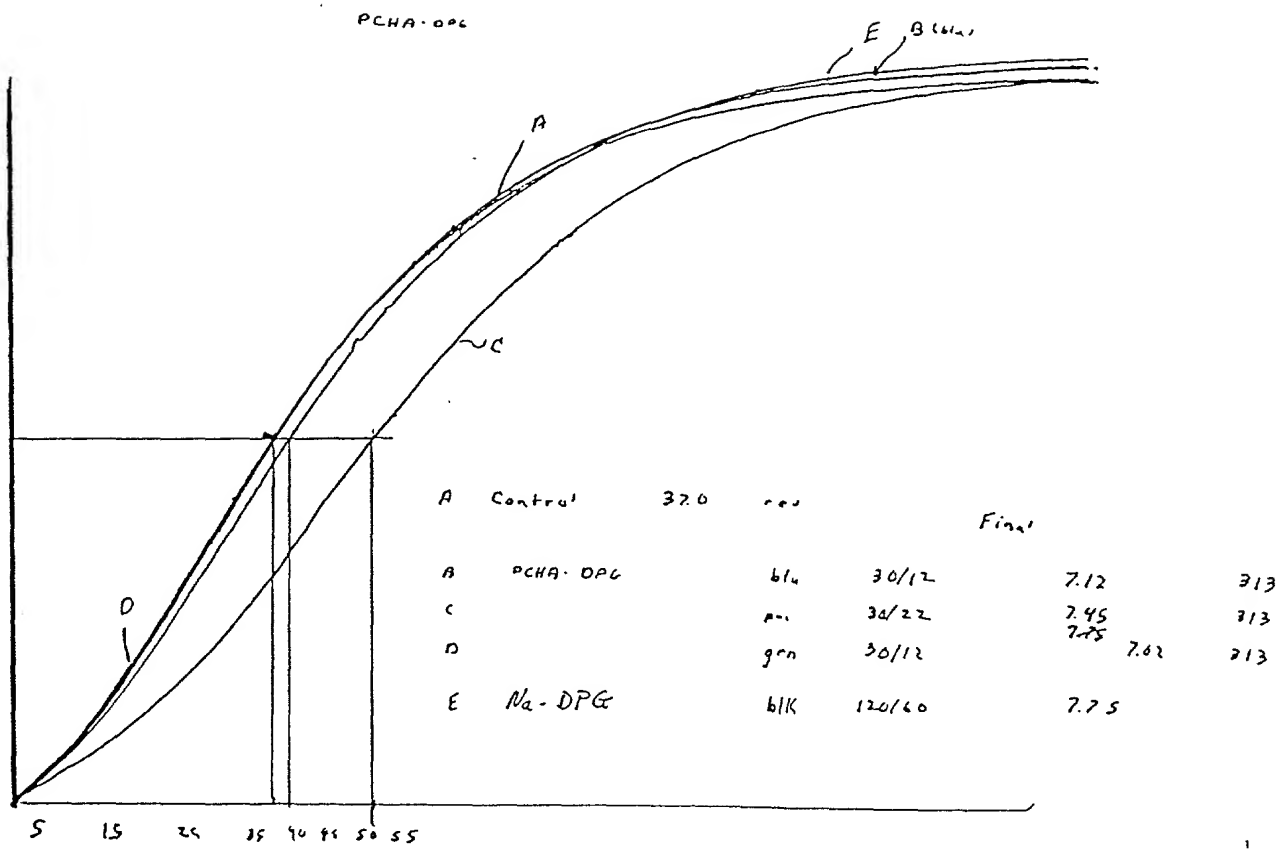


Fig 7 Oxygen Dissociation Curves of **Whole Blood** treated with a solution of pentacyclohexylammonium-2,3 diphosphogliceric acid (PCHA-DPG) and Sodium salt of DPG (PNa-DPG).

A: Control (25µL WHOLE BLOOD).
$P_{50} = 37.0$

C: 75µL Whole Blood incubated (2-5 min) with 200µL 30mM PCHA-DPG. After incubation the system was washed 4X and 15µL RBC were used for measurement of the Hb-O_2 dissociation curve at 37°C.
$P_{50} = 50.5$

E: 75µL Whole Blood incubated (2-5 min) with 200µL 30mM PNa-DPG.
$P_{50} = 38.2$

Incubation Time: 2-5 min at 37°C. All Experiments were conducted with **Whole Blood.**

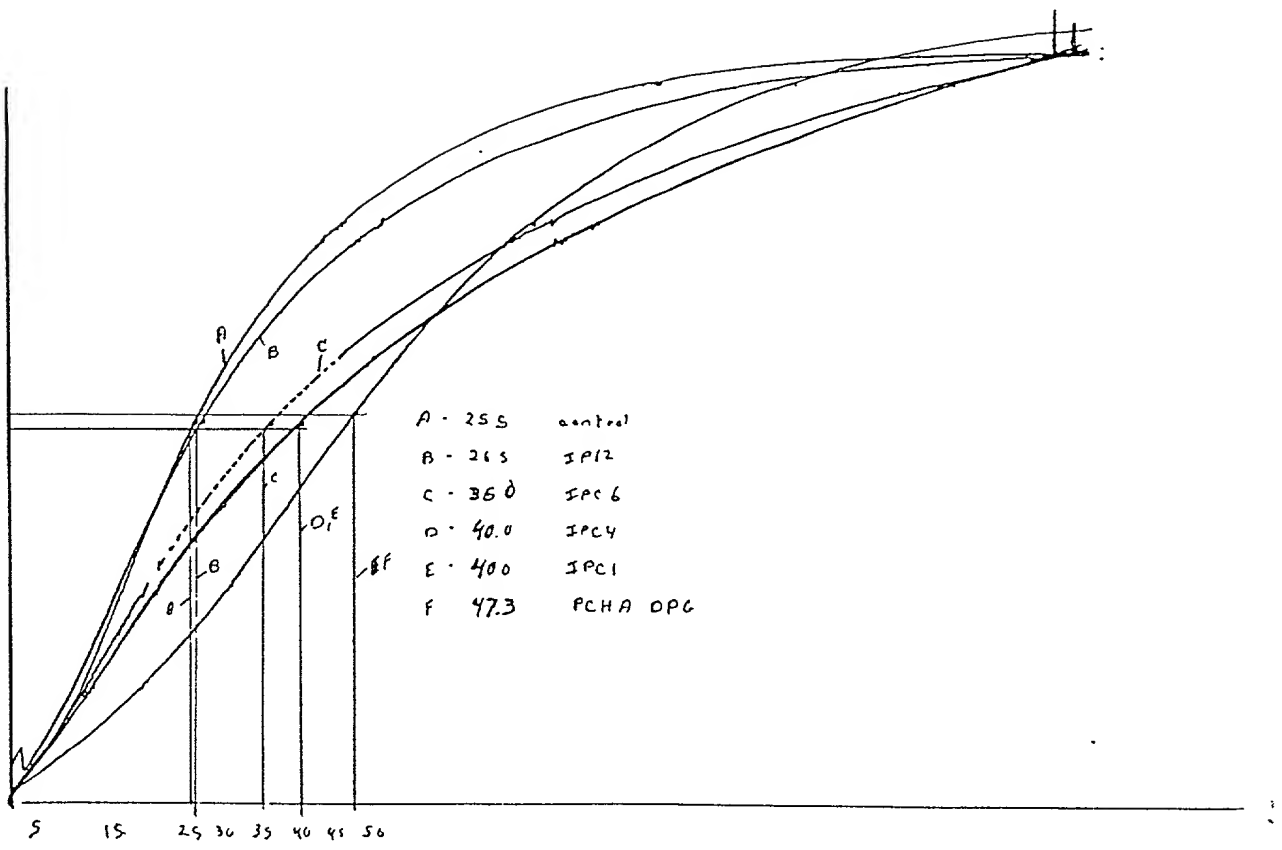


Fig 6 Oxygen Dissociation Curves of **Whole Blood** treated with a solution of pentacyclohexylammonium-2,3 diphosphogliceric acid (PCHA-DPG)

A: Control (25µL WHOLE BLOOD).
$P_{50} = 25.5$

F: 75µL Whole Blood incubated (2-5 min) with 200µL 30mM PCHA-DPG. After incubation the system was washed 4X and 15µL RBC were used for measurement of the Hb-O_2 dissociation curve at 37°C.
$P_{50} = 47.3$

Incubation Time: 2-5 min at 37°C. All Experiments were conducted with **Whole Blood.**

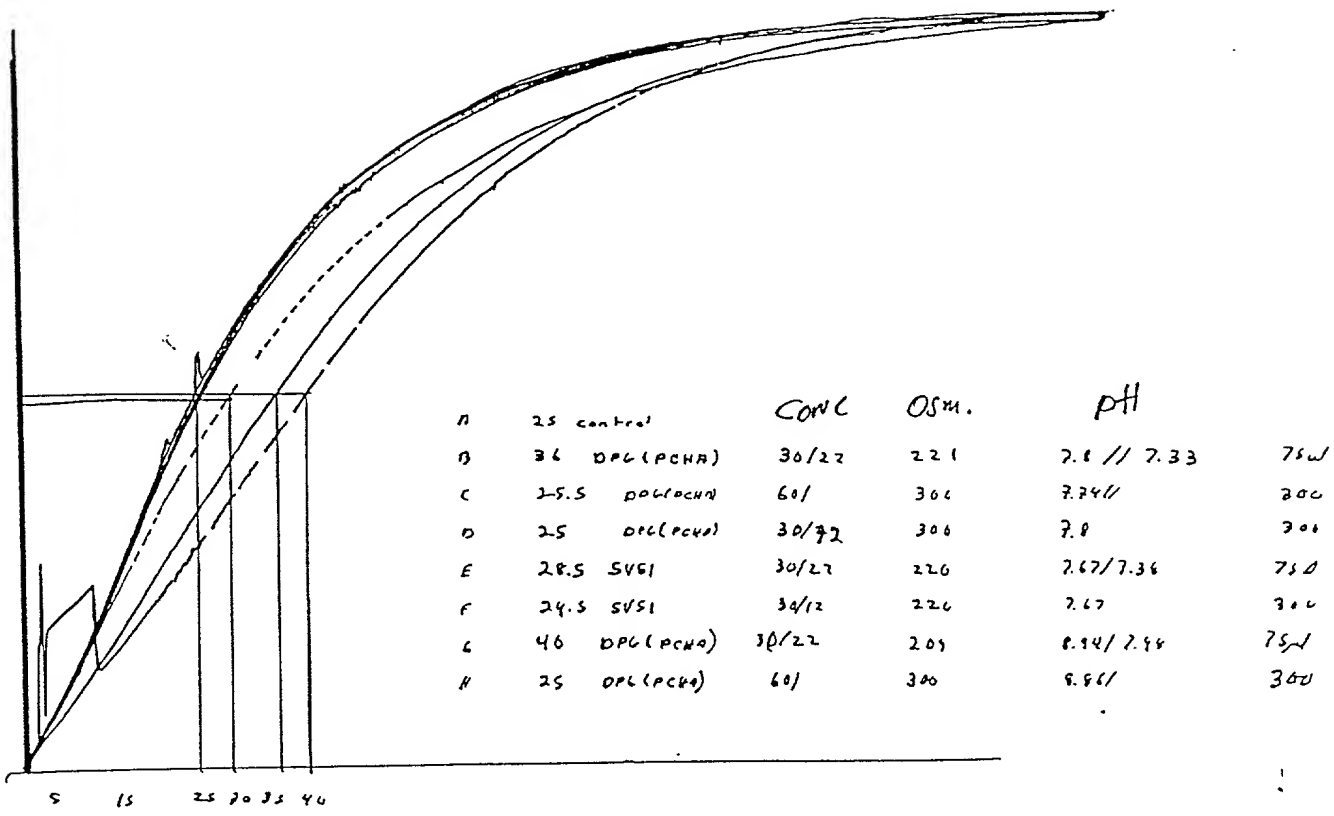


Fig9. Oxygen Dissociation Curves of **Whole Blood** treated with a solution of pentacyclohexylammonium-2,3 diphosphogliceric acid (PCHA-DPG)

A: Control (25µL WHOLE BLOOD).
$P_{50} = 25.0$

B: 75µL Whole Blood incubated (2-5 min) with 200µL 30mM PCHA-DPG. After incubation the system was washed 4X and 15µL RBC were used for measurement of the Hb-O_2 dissociation curve at 37°C.
$P_{50} = 36.0$

G: 75µL Whole Blood incubated (2-5 min) with 200µL 30mM PCHA-DPG..
$P_{50} = 40.0$

Incubation Time: 2-5 min at 37°C. All Experiments were conducted with **Whole Blood.**

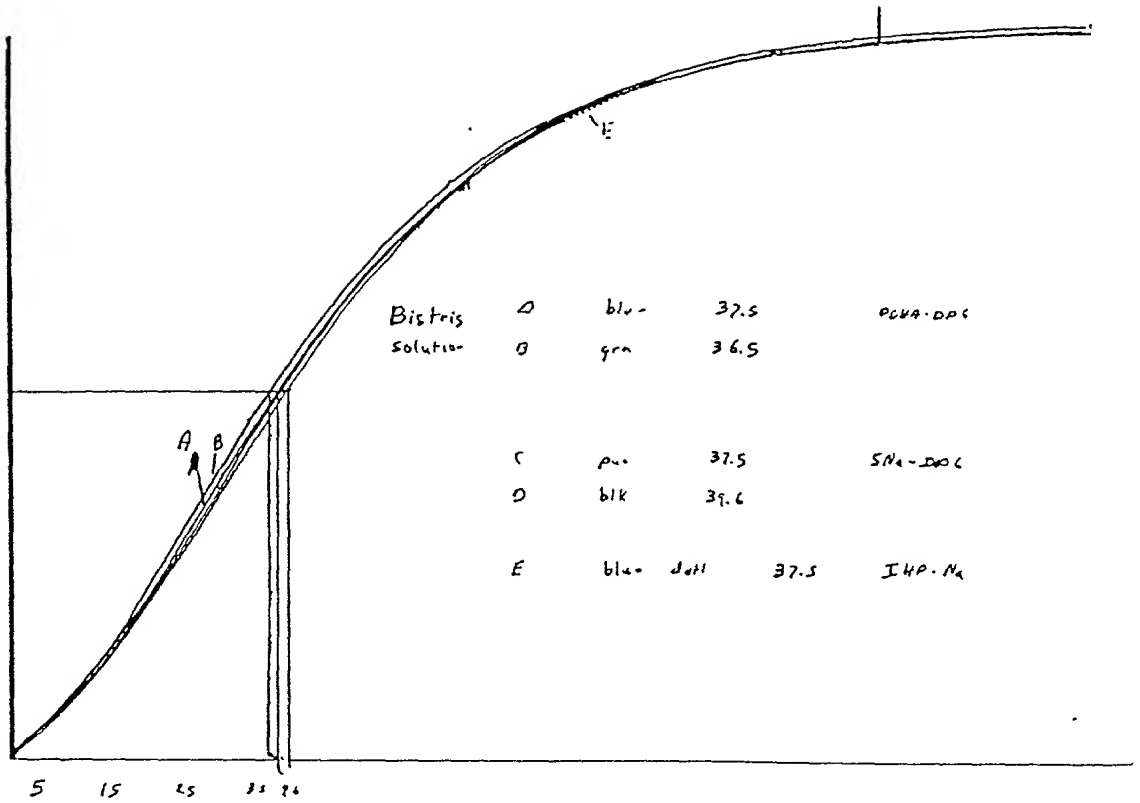


Fig 10 Oxygen Dissociation Curves of **Whole Blood** treated with a solution of Sodium Salts of DPG and IHP.

A: Control (25µL WHOLE BLOOD).
$P_{50} = 37.0$

C: 75µL Whole Blood incubated (2-5 min) with 200µL 30mM PNa-DPG. After incubation the system was washed 4X and 15µL RBC were used for measurement of the Hb-O_2 dissociation curve at 37°C.
Hypotonic. Osm: 163mOsM
$P_{50} = 37.5$

D: 75µL Whole Blood incubated (2-5 min) with 200µL 30mM PNa-DPG.
Isotonic. Osm: 321 mOsM
$P_{50} = 39.6$

E: 75µL Whole Blood incubated (2-5 min) with 200µL 30mM Na-IHP
Hypotonic. Osm: 185mOsM
$P_{50} = 37.5$

Incubation Time: 2-5 min at 37°C. All Experiments were conducted with **Whole Blood.**

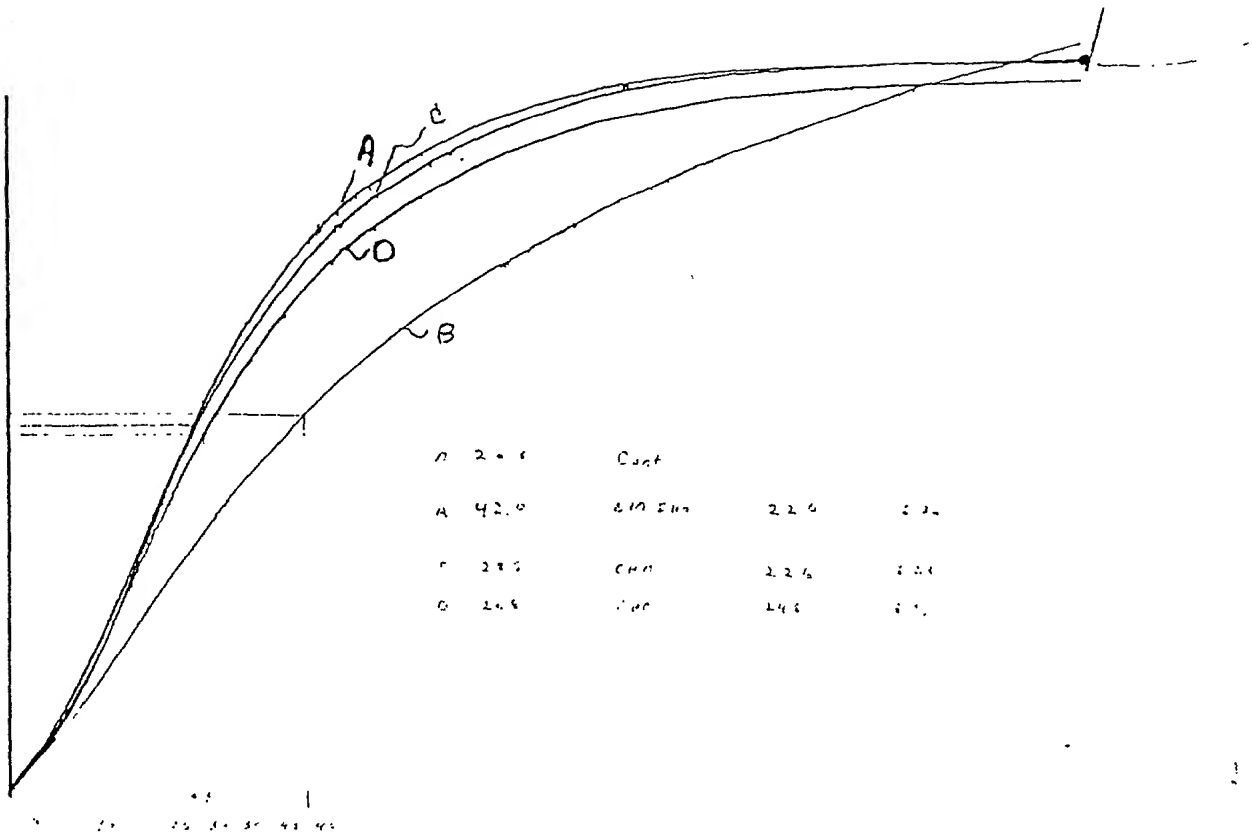


Fig 11 Oxygen Dissociation Curves of Whole Blood treated with a solution of Cyclohexylammonium (CHA) and CHA salt of IHP

A: Control (25µL WHOLE BLOOD).
$P_{50} = 26.8$

B: 75µL Whole Blood incubated (2-5 min) with 200µL 30mM CHA-IHP. After incubation the system was washed 4X and 15µL RBC were used for measurement of the Hb-O_2 dissociation curve at 37°C.
$P_{50} = 42.0$

C: 75µL Whole Blood incubated (2-5 min) with 200µL 30mM CHA.
$P_{50} = 28.5$

D: 75µL Whole Blood incubated (2-5 min) with 200µL 30mM CHA.
$P_{50} = 26.8$

Incubation Time: 2-5 min at 37°C. All Experiments were conducted with Whole Blood.

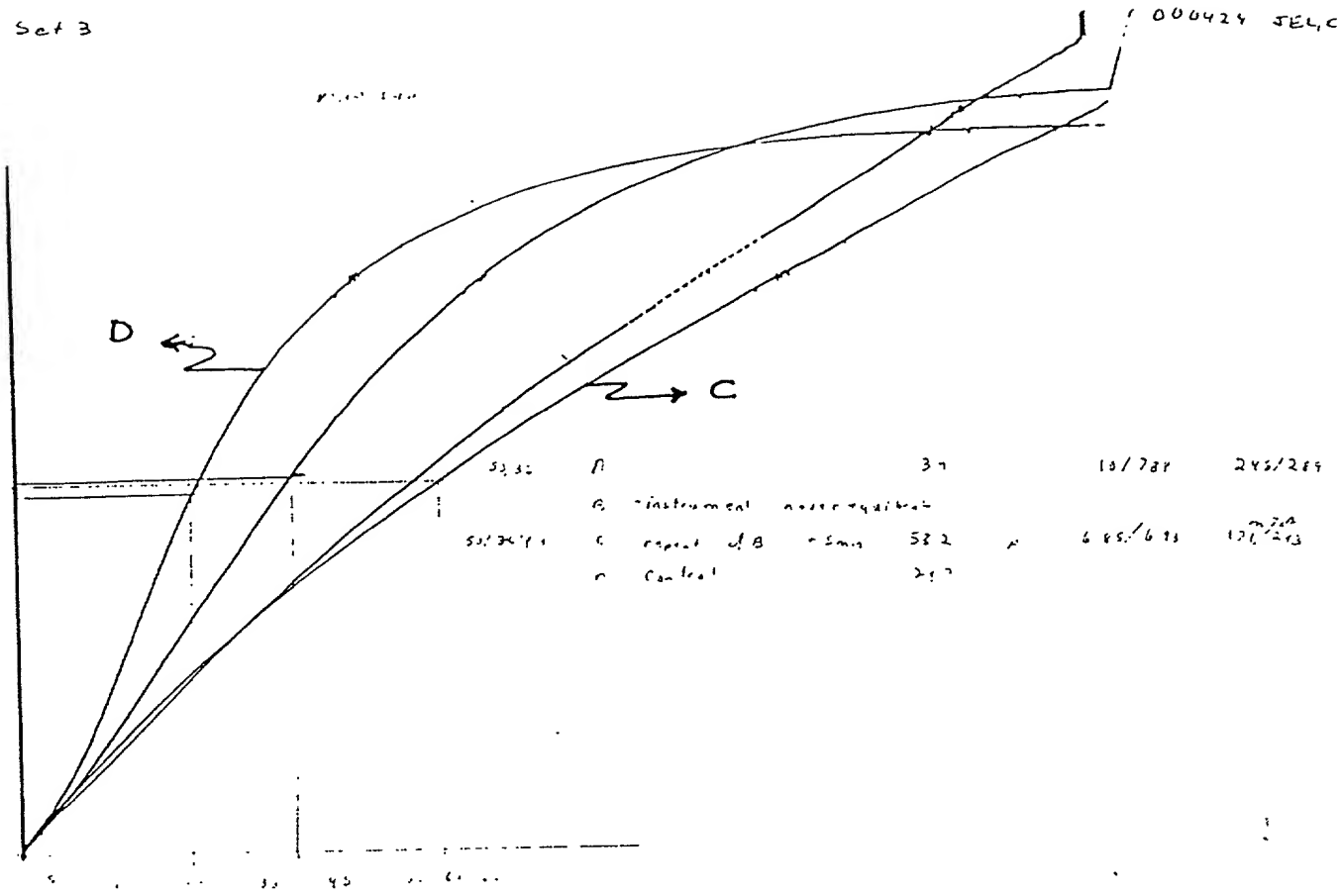
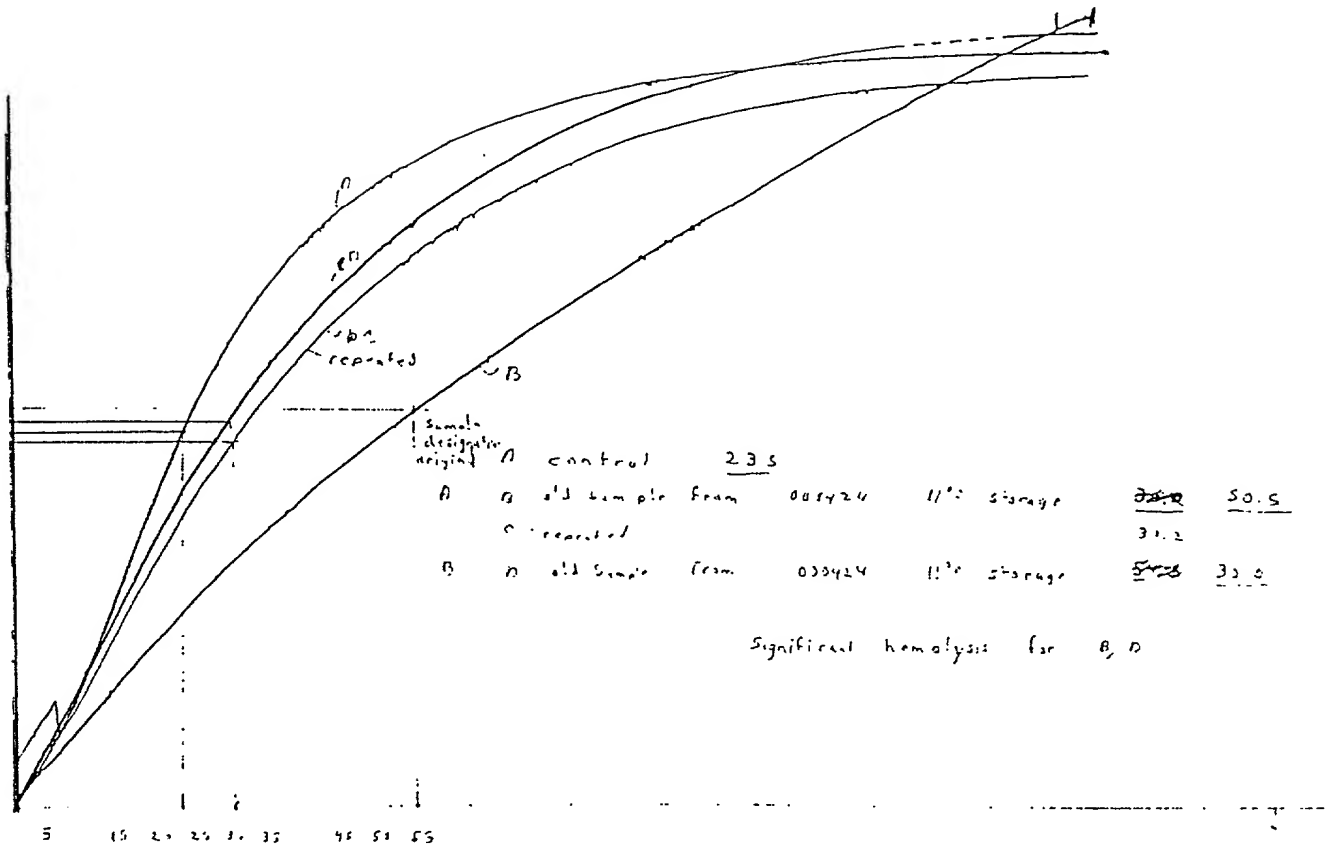


Fig 12 Oxygen Dissociation Curves of **Whole Blood** treated with a solution of Cyclohexylammonium-Inositol Hexaphosphate (CHA-IHP)

D: Control (25µL WHOLE BLOOD).
$P_{50} = 24.7$

C: 75µL Whole Blood incubated (2-5 min) with 200µL 30mM CHA-IHP. After incubation the system was washed 4X and 15µL RBC were used for measurement of the Hb-O_2 dissociation curve at 37°C.
$P_{50} = 58.2$

Incubation Time: 2-5 min at 37°C. All Experiments were conducted with **Whole Blood.**



Fig/3 Oxygen Dissociation Curves of **Whole Blood** treated with a solution of Cyclohexylammonium-Inositol Hexaphosphate (CHA-IHP)

A: Control (25µL WHOLE BLOOD).
$P_{50} = 23.5$

C: 75µL Whole Blood incubated (2-5 min) with 200µL 30mM CHA-IHP. After incubation the system was washed 4X . Whole Blood Cell Pellet was stored for 48 hrs at 4-8°C and 15µL RBC were used for measurement of the Hb-O_2 dissociation curve at 37°C.
$P_{50} = 50.5$

Incubation Time: 2-5 min at 37°C. All Experiments were conducted with **Whole Blood.**

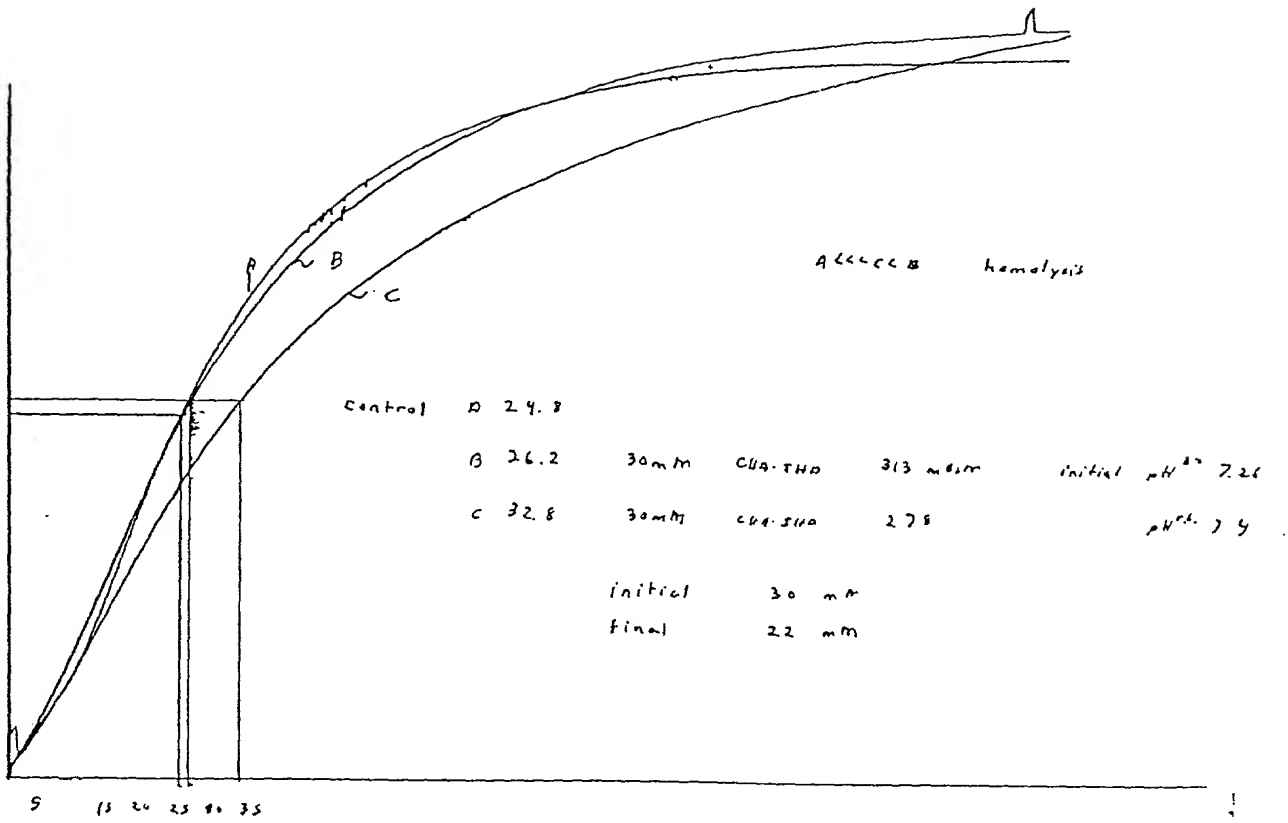


Fig 4 Oxygen Dissociation Curves of Whole Blood treated with a solution of Cyclohexylammonium-Inositol Hexaphosphate (CHA-IHP)

A: Control (25µL WHOLE BLOOD).
$P_{50} = 24.8$

C: 75µL Whole Blood incubated (2-5 min) with 200µL 30mM CHA-IHP. After incubation the system was washed 4X and 15µL RBC were used for measurement of the Hb-O_2 dissociation curve at 37°C.
$P_{50} = 32.8$

Incubation Time: 2-5 min at 37°C. All Experiments were conducted with **Whole Blood.**